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Leaf rehydration capacity: Associations with other indices of drought tolerance and environment

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Abstract

Clarifying the mechanisms of leaf and whole plant drought responses is critical to predict the impacts of ongoing climate change. The loss of rehydration capacity has been used for decades as a metric of leaf dehydration tolerance but has not been compared with other aspects of drought tolerance. We refined methods for quantifying the percent loss of rehydration capacity (PLRC), and for 18 Southern California woody species, we determined the relative water content and leaf water potential at PLRC of 10%, 25%, and 50%, and, additionally, the PLRC at important stages of dehydration including stomatal closure and turgor loss. On average, PLRC of 10% occurred below turgor loss point and at similar water status to 80% decline of stomatal conductance. As hypothesized, the sensitivity to loss of leaf rehydration capacity varied across species, leaf habits, and ecosystems and correlated with other drought tolerance traits, including the turgor loss point and structural traits including leaf mass per area. A new database of PLRC for 89 species from the global literature indicated greater leaf rehydration capacity in ecosystems with lower growing season moisture availability, indicating an adaptive role of leaf cell dehydration tolerance within the complex of drought tolerance traits.

KEYWORDS

drought tolerance, leaf, leaf damage, lethal water status

1 | INTRODUCTION

Given global climate change, under which droughts are projected to increase in frequency and severity in many locations (Carrão, Naumann, & Barbosa, 2017; Dai, 2011, 2013; Duffy, Brando, Asner, & Field, 2015; MacDonald, 2010; Yoon et al., 2015), the mechanistic quantification of physiological tolerance of drought is critical for prediction of species responses. The maintenance of leaf function during drought depends on the properties of all plant organs (e.g., including leaf stomatal closure, stem resistance to embolism, and rooting depth) and of the whole plant (e.g., including biomass allocation to roots and resprouting; Larcher, 2003; Jones, 2013). Ultimately, each of these traits can "delay" the stress associated with soil or atmospheric drought, that is, to protect the photosynthetic leaf tissue from dehydration. The aim of this work was to quantify the variation across ecosystems in leaf rehydration capacity, the most commonly used index of whole-leaf tissue dehydration tolerance, and to test its linkage with other leaf drought tolerance traits.

Leaf rehydration capacity, that is, the potential for recovery after dehydration, has been measured for diverse species for nearly 60 years (Oppenheimer, 1963). According to this approach, excised leaves are dehydrated to a range of relative water contents (RWCs; see Table 1 for legend of symbols) and then rehydrated with petioles immersed in water until net water uptake ceases. Leaves subjected to greater dehydration progressively lose the capacity to rehydrate, and one can thus quantify the RWC corresponding to a given percent loss of rehydration capacity (PLRC). Oppenheimer associated a PLRC of 10% in *Nerium oleander* with loss of leaf cell viability and death as assessed by vital staining and cross-sectional anatomy of dehydrated leaves and designated this threshold ($PLRC_{10}$) as the "sublethal water

All data are included as supplement and will be deposited into larger databases.

TABLE 1 Legend of trait symbols and their units

Symbol	Trait	Units
Thresholds for leaf viability loss		
$RWC@PLRC_{10}$	Leaf relative water content at which 10% of rehydration capacity is lost	%
$PLRC_{25}$	25% of rehydration capacity is lost	%
$PLRC_{50}$	50% of rehydration capacity is lost	%
$\Psi@PLRC_{10}$	Leaf water potential at which 10% of rehydration capacity is lost	MPa
$PLRC_{25}$	25% of the capacity to rehydrate is lost	MPa
$PLRC_{50}$	50% of the capacity to rehydrate is lost	MPa
Leaf viability loss at given key stages of leaf dehydration		
$PLRC@SC_{80}$	% of rehydration capacity lost at 80% stomatal closure	%
SC_{50}	50% stomatal closure	%
π_{tlp}	Turgor loss point	%
−2 MPa	Water potential of −2 MPa	%
RWC_{70}	70% relative water content	%
Leaf water relation traits		
Ψ_{SC50}	Leaf water potential at 50% loss of stomatal conductance	MPa
Ψ_{SC80}	Leaf water potential at 80% loss of stomatal conductance	MPa
RWC_{SC50}	Relative water content at 50% loss of stomatal conductance	%
RWC_{SC80}	Relative water content at 80% loss of stomatal conductance	%
g_{min}	Minimum epidermal conductance	$mmol \cdot m^{-2} \cdot s^{-1}$
π_o	Leaf osmotic potential at full turgor	MPa
π_{tlp}	Leaf water potential (or osmotic potential) at turgor loss point	MPa
RWC_{tlp}	Relative water content at turgor loss point	%
ε	Modulus of elasticity	MPa
C_{ft}	Capacitance at full turgor	MPa^{-1}
Leaf structure and composition		
LT	Leaf thickness	mm
LA	Leaf area	cm^2
LD	Leaf density	$g \cdot m^{-3}$
LMA	Leaf mass per area	$g \cdot m^{-2}$
SWC	Saturated water content	$g \cdot g^{-1}$
Climate variables		
AI	Aridity index	—
PET	Potential evapotranspiration	mm
MAT	Mean annual temperature	°C
MAP	Mean annual precipitation	mm

deficit" or "permanent turgor loss point" (Oppenheimer, 1963; Oppenheimer & Leshem, 1966). Subsequent studies of diverse ecosystems quantified this index to compare coexisting species and achieved insights regarding variation in drought tolerance within and among specific ecosystems (Table S7; Oppenheimer, 1963; Rychnovská-Soudková, 1963; Oppenheimer & Leshem, 1966; Rychnovska, 1967; Weinberger, Romero, & Oliva, 1972; Buckley, Corlett, & Grubb, 1980; Florineth, 1980; Peace & Macdonald, 1981; Rambousková, 1981; Hinckley, Duhme, Hinckley, & Richter, 1983; Goldstein, Meinzer, & Monasterio, 1985; Kapos & Tanner, 1985; Park, 1989; Alexandre, 1991; Burghardt & Riederer, 2003; Burghardt, Burghardt, Gall, Rosenberger, & Riederer, 2008). The $PLRC_{10}$ has practical advantages over other indices of leaf dehydration tolerance, such as the visually estimated "lethal leaf water potential," which is difficult to ascertain objectively (Augé, Duan, Croker, Witte, & Green, 1998; Augé, Stodola, Moore, Klingeman, & Duan, 2003; Brodribb, Bowman, Nichols, Delzon, & Burlett, 2010; Chapman & Augé, 1994; Kubikova, Jennifer, Bonnie, Michael, & Augé, 2001; Kursar et al., 2009). Quantifying PLRC is logistically simpler and complementary to other indices of leaf dehydration tolerance such as the water potentials corresponding to the decline of chlorophyll fluorescence (Lamont & Lamont, 2000), the loss of leaf or stem hydraulic conductance (e.g., Choat et al., 2012; Li et al., 2016; Scoffoni, McKown, Rawls, & Sack, 2012), the inability to recover stomatal conductance or photosynthesis (Augé et al., 2002; Brodribb, Holbrook, Edwards, & Gutiérrez, 2003; Engelbrecht, Tyree, & Kursar, 2007; Tyree, Engelbrecht, Vargas, & Kursar, 2003), the leakage of solutes (Demidchik et al., 2014; Leopold, Musgrave, & Williams, 1981), or the shedding of leaves (Tyree, Cochard, Cruiziat, Sinclair, & Ameglio, 1993). The PLRC also has the advantage of analogy to estimates of lethal doses of stresses and toxins in plants and animals (e.g., LD50; Trevan, 1927; Zbinden & Flury-Roversi, 1981; Meier & Theakston, 1986; Kursar et al.,

2009). However, no studies to our knowledge have placed thresholds for PLRC within the sequence of leaf drought response, that is, in relation to stomatal closure or turgor loss, or tested the potential correlation across species of thresholds for PLRC with other drought tolerance indices.

We focused on 18 native species of Southern California desert, chaparral, and woodland ecosystems (Table 2). We tested hypotheses for the variation across species in PLRC and its association with leaf structure, habit, and composition, and with species' ecological preferences and the climate of their native ranges. We hypothesized that (a) substantial PLRC would occur only after virtually complete stomatal closure and turgor loss point and that (b) leaves would be more sensitive to PLRC for species of moister ecosystems or climatic ranges, and for deciduous than evergreen species (Table 3) and that (c) PLRC would correlate across species with other drought tolerance traits, including the leaf water potential thresholds for 50% and 80% stomatal closure and turgor loss, and the osmotic potential at full turgor, and with structural "stress tolerance" or "succulence" traits such leaf mass per area (LMA), leaf thickness (LT), leaf density (LD), and saturated water content (SWC; de la Riva, Olmo, Poorter, Ubera, & Villar, 2016; Dedio, 1975; Hallik, Niinemets, & Wright, 2009; Ogburn & Edwards, 2012; Oppenheimer, 1959; Reich, 2014; Reich, Walters, & Ellsworth, 1997; Violle et al., 2007). In addition, we compiled previously published data for PLRC of diverse species to analyse variation among growth forms, leaf habits, and ecosystems and hypothesized (d) that leaves would have greater leaf dehydration tolerance in grasses than trees or shrubs, or evergreen than cold- or drought-deciduous species and in drier than moister ecosystems.

2 | METHODS

2.1 | Plant material

We grew 18 tree and shrub species of Southern California (Table 2) in a greenhouse common garden at the UCLA Plant Growth Center from August 2012 to April 2016, and experiments were carried out from April to October 2015. This common garden approach minimized environmental variation that would have driven plastic responses in the field across species of different native ecosystems. Nine individual seedlings of each species were acquired in 3.8-L pots (Tree of Life Nursery, San Juan Capistrano, CA). Plants were acclimated 12–18 months prior to initial measurements to establish similar external conditions across individuals and species, and to ensure canopies of mature leaves. Plants were carefully monitored for root expansion and repotted when roots filled the pots. Given the species variation in natural history, phenology and growth rate, 19- to 38-L pots were used, as appropriate for each species to minimize stress and accommodate species of different sizes and intrinsic growth rates (Poorter, Buhler, van Dusschoten, Climent, & Postma, 2012). Potting soil (18.75% washed plaster sand, 18.75% sandy loam, 37.5% grower grade peat moss, 12.5% horticultural-grade perlite, 12.5% coarse vermiculite; Therm-O-Rock West, Inc., Chandler, AZ) was autoclaved prior to use.

The plants were randomized within each of nine blocks containing one individual of each species and placed on four greenhouse benches in two greenhouse rooms. Plants were irrigated each second day with 200–250 ppm 20:20:20 NPK fertilizer. Daily irradiance ranged up to 1,400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (LI-250 Light Meter; Li-Cor Biosciences, Lincoln,

TABLE 2 Trees and shrubs common to Southern California used in our analyses

Species	Family	Leaf habit	Ecosystem	Growth form	RWC@ $PLRC_{10}$	RWC@ $PLRC_{25}$	PLRC@π_{tlp}	PLRC@−2 MPa	PLRC @SC_{50}	PLRC @SC_{80}
Cleome isomeris	Capparaceae	E	D	S	49.2	38.2	0.0	22.2	na	na
*Encelia californica**	Asteraceae	D	D	S	69.3	54.6	na	na	na	na
Encelia farinosa	Asteraceae	D	D	S	78.8	70.6	1.2	30.0	6.4	21.4
Cercocarpus betuloides	Rosaceae	E	C	S	44.6	34.5	0.0	0.0	0.0	22.1
*Ceanothus spinosus**	Rhamnaceae	E	C	S	30.5	24.1	na	na	na	na
Malosma laurina	Anacardiaceae	E	C	T	76.6	61.0	0.7	0.0	0.0	26.1
*Prunus ilicifolia**	Rosaceae	E	C	T	79.6	61.0	10.5	9.6	8.1	54.0
*Quercus agrifolia**	Fagaceae	E	C	T	65.3	42.0	4.7	0.0	3.5	11.4
*Quercus berberidifolia**	Fagaceae	E	C	T	35.4	24.1	0.0	0.0	0.0	0.0
Rhus integrifolia	Anacardiaceae	E	C	S	61.9	47.9	0.0	0.0	na	na
*Salvia leucophylla**	Lamiaceae	E	C	S	43.6	34.4	0	21.7	na	na
Alnus rhombifolia	Betulaceae	D	W	T	79.9	61.6	21.7	39.1	17.0	20.9
Betula occidentalis	Betulaceae	D	W	T	85.5	66.9	8.8	12.3	2.9	8.5
*Cercis occidentalis**	Fabaceae	D	W	S	69.9	55.8	10.8	7.1	0.0	0.2
Fraxinus dipetala	Oleaceae	D	W	S	80.2	57.5	17.8	18.2	1.6	3.5
Platanus racemosa	Platanaceae	D	W	T	85.1	69.1	14.0	19.6	13.4	23.6
Quercus engelmannii	Fagaceae	E	W	T	70.3	51.4	10.9	7.3	na	na
*Umbellularia californica**	Lauraceae	E	W	T	80.1	62.6	4.0	2.2	0.0	7.8

Note. Leaf habit (E = evergreen, D = deciduous), vegetation type (D = desert, C = chaparral, W = woodland), and growth form (S = shrub, T = tree). Relative water content at 10% and 25% loss of rehydration capacity (RWC@PLRC; %) and the percent loss of rehydration capacity at turgor loss point (PLRC@π_{tlp}), at a bulk leaf water potential of −2 MPa (PLRC@−2 MPa), and 50% and 80% loss of stomatal conductance (PLRC@SC_{50}; PLRC@SC_{80}) are presented for each species. Asterisk indicates species used in timed rehydration experiments.

TABLE 3 Leaf dehydration tolerance, expressed as the thresholds in relative water content (RWC) and bulk leaf water potential (Ψ) for loss of 10%, 25%, or 50% rehydration capacity (PLRC) and the PLRC corresponding to given key stages of leaf dehydration, that is, at 80% and 50% stomatal closure (RWC_{SC80} and RWC_{SC50}), at turgor loss point (PLRC@π_{tlp}), and at −2 MPa (PLRC@−2 MPa); and other drought tolerance parameters and leaf structural and compositional traits, the relative water content at turgor loss point (RWC_{tlp}) and bulk leaf water potential at turgor loss point (π_{tlp}), osmotic potential at full turgor (π_o), water potential at 80% and 50% stomatal closure (Ψ_{SC80} and Ψ_{SC50}), minimum epidermal conductance (g_{min}; mmol·m^{-2}·s^{-1}), leaf saturated water content (SWC; g·g^{-1}), and leaf mass per area (LMA; g·m^{-2})

	Leaf habit				Ecosystem			
Trait	Deciduous	Evergreen	*P*	Hypothesis	Chaparral	Woodland	*P*	Hypothesis
Thresholds for leaf viability loss								
$RWC@PLRC_{10}$	**78.1 ± 2.9**	**59.1 ± 6.5**	**0.02**	↑D	**54.7 ± 6.6**	**76.8 ± 3.3**	**0.03**	↑W
$RWC@PLRC_{25}$	**62.3 ± 2.7**	**43.7 ± 4.7**	**0.009**	↑D	**41.1 ± 5.2**	**60.7 ± 3.6**	**0.03**	↑W
$RWC@PLRC_{50}$	**35.5 ± 4.8**	**20.1 ± 3.5**	**0.03**	↑D	18.5 ± 4.0	30.7 ± 4.7	0.08	↑W
$\Psi@PLRC_{10}$	**−1.5 ± 0.2**	**−2.8 ± 0.3**	**0.01**	↑D	−3.1 ± 0.4	−1.8 ± 0.4	0.14	↑W
$\Psi@PLRC_{25}$	**−2.4 ± 0.3**	**−3.9 ± 0.6**	**0.05**	↑D	−4.3 ± 0.6	−2.7 ± 0.4	0.11	↑W
$\Psi@PLRC_{50}$	−3.2 ± 0.3	−6.9 ± 1.8	0.06	↑D	−8.4 ± 2.3	−3.9 ± 0.7	0.11	↑W
Leaf viability loss at given key stages of leaf dehydration								
$PLRC@SC_{50}$	6.9 ± 2.9	1.9 ± 1.1	0.14	↑D	2.3 ± 1.3	5.8 ± 4.6	0.62	↑W
$PLRC@SC_{80}$	13.0 ± 4.2	20.2 ± 6.4	0.46	↑D	22.7 ± 7.1	10.8 ± 5.5	0.45	↑W
PLRC@π_{tlp}	**12.4 ± 2.9**	**3.1 ± 1.5**	**0.02**	↑D	**2.3 ± 1.4**	**12.6 ± 3.4**	**0.04**	↑W
PLRC@−2 MPa	**21.0 ± 4.8**	**6.3 ± 3.0**	**0.002**	↑D	**4.5 ± 3.0**	**15.1 ± 7.1**	**0.03**	↑W
Other drought response parameters								
RWC_{tlp}	75.7 ± 3.7	73.2 ± 3.0	0.90	↑D	71.8 ± 3.3	75.3 ± 5.4	0.62	↑W
RWC_{SC80}	77.7 ± 3.9	56.9 ± 7.4	0.08	↑D	51.8 ± 7.2	79.4 ± 5.3	0.08	↑W
RWC_{SC50}	85.3 ± 3.6	81.5 ± 3.2	0.50	↑D	79.0 ± 3.0	87.5 ± 5.3	0.34	↑W
π_{tlp}	**−1.8 ± 0.1**	**−2.3 ± 0.2**	**0.002**	↑D	**−2.4 ± 0.2**	**−2.0 ± 0.2**	**0.01**	↑W
π_o	**−1.4 ± 0.1**	**−1.7 ± 0.2**	**0.01**	↑D	−1.7 ± 0.2	−1.6 ± 0.2	0.13	↑W
Ψ_{SC80}	**−1.5 ± 0.2**	**−3.3 ± 0.5**	**0.02**	↑D	−3.4 ± 0.6	−1.7 ± 0.4	0.09	↑W
Ψ_{SC50}	−1.0 ± 0.2	−1.8 ± 0.2	0.06	↑D	−1.9 ± 0.2	−1.0 ± 0.3	0.06	↑W
g_{min}	3.5 ± 0.6	4.9 ± 1.4	0.86	↑D	3.8 ± 0.7	3.3 ± 1.1	0.74	↑W
Leaf structure and composition								
SWC	**3.4 ± 0.6**	**1.9 ± 0.4**	**0.01**	↑D	**1.7 ± 0.2**	**2.2 ± 0.3**	**<0.001**	↑W
LMA	**54.9 ± 4.6**	**109.7 ± 11.7**	**<0.001**	↑E	**119.9 ± 11.7**	**69.8 ± 12.0**	**<0.001**	↑C

Note. Mean values are presented for leaf habits (evergreen vs. deciduous), growth form (tree vs. shrub), and ecosystem (chaparral vs. woodland; desert species excluded due to low replication). Subscripts indicate parameters as a percent loss of water content recovery (10, 25, or 50), at turgor loss points (tlp), or at 50% or 80% stomatal closure (SC_{50} or SC_{80}). Means ± standard errors are reported for each group with *P* values reported for one-way multivariate analyses of variance run on each group of traits (e.g., all RWC@PLRC traits). Traits that varied significantly across groups are shown in bold. RWC means are reported for each group in %, and Ψ in MPa.

NE, USA), and mean minimum, mean, and maximum values for temperature were respectively 22.1°C, 23.9°C, and 25.2°C and values for relative humidity were respectively 47.3%, 60.1%, and 72.8% over the course of our experiments (HOBO Micro Station with Smart Sensors; Onset, Bourne, MA, USA).

Prior to rehydration experiments, whole plants were drought hardened by watering to field capacity and then suspending watering until visible wilting was observed; leaf water potentials were measured on leaves for six individuals per species and ranged −1.1 to −4.3 MPa (Plant Moisture Stress pressure chamber model 1000; PMS Instrument Co., Albany, OR, USA).

For all measurements, including rehydration curves, pressure–volume curves, stomatal responses, and minimum cuticular conductance, individual leaves were sampled using a razor blade and immediately sealed in Whirl-Pak bags that had been exhaled into and then placed in a second bag filled with wet paper for transport to the laboratory. Leaves were rehydrated overnight at least 8 hr (see Section 3.1) in a 15- or 50-ml centrifuge tube (depending upon leaf size) with only the petiole submerged in deionized water, wrapped in wet paper, and covered with two plastic bags.

2.2 | Testing the time required to complete leaf rehydration

The published literature shows that a wide range of species achieve maximum rehydration after 3–6 hr (Arndt, Irawan, & Sanders, 2015; Garnier, Shipley, Roumet, & Laurent, 2001). To confirm the rehydration time needed for net water uptake to cease, we tested the time course of rehydration for eight species (indicated with asterisks in Table 2) selected to span a range of drought sensitivity (turgor loss points ranging from −1.8 to −3.0 MPa), leaf habits, and native ecosystems. For each species, eight to 17 leaves were rehydrated overnight, weighed for saturated mass, and then dehydrated over a fan. Then, leaves were rehydrated with petioles submerged in deionized water in tubes, wrapped in wet paper, and covered with plastic bags. Leaves were dried with paper towels; weighed after 8,

12, and 24 hr of rehydration; and finally oven dried at 70°C for 72 hr to obtain dry mass. For each leaf, we determined the relative water content as

$$\mathrm{RWC} = \frac{\mathrm{fm}-\mathrm{dm}}{\mathrm{sm}-\mathrm{dm}}, \quad (1)$$

where fm is the fresh mass of the dehydrated leaf, sm is the initial saturated mass, and dm is the leaf mass after oven drying.

We tested differences in the recovery of RWC at 8, 12, and 24 hr of rehydration.

2.3 | Determining leaf rehydration capacity and its loss

We tested the rehydration capacity of leaves after bench dehydration. For each of the 18 study species, after initial overnight rehydration of sampled shoots, 16–38 leaves were measured for initial mass (±0.01 mg; MS205DU; Mettler Toledo, OH) and lamina area (using a flatbed scanner, Epson Perfection 4490 Photo scanner; 600 dots per inch; and ImageJ software version 1.42q; National Institutes of Health). Leaves were bench dried from 5 min to >9 days before measuring mass again. The dehydrated leaves then had their petioles submerged under deionized water in tubes, and the tubes were wrapped in moist paper and covered with plastic. Leaves were weighed for determination of water content after >8-hr rehydration, on the basis of the rehydration time experiments (described in previous section; see Section 3 below), and then dry mass was determined after oven drying for >72 hr at 70°C.

For each species, we plotted the relationship of PLRC against dehydrated RWC. We determined the RWC and leaf water potentials at given PLRC values such that

$$\mathrm{PLRC}_x = 100\%-\mathrm{SWC}_{\mathrm{rehy}}/\mathrm{SWC}_{\mathrm{orig}}, \quad (2)$$

where $\mathrm{SWC}_{\mathrm{rehy}}$ is the SWC after dehydrating leaves on the bench and rehydrating overnight and $\mathrm{SWC}_{\mathrm{orig}}$ is the initial SWC of leaves rehydrated from the greenhouse and weighed before bench dehydration.

Ordinary least squares regression was used to determine the relationship of PLRC to RWC, and lines were fitted for leaves with PLRC > 10%. From these regressions, we calculated two types of parameters. Firstly, we calculated the leaf water status (RWC and leaf water potential, Ψ_{leaf}) at given PLRC thresholds, that is, at PLRC of 10%, 25%, and 50% (PLRC_{10}, PLRC_{25}, and PLRC_{50}, respectively). These parameters are analogous to those generally used for estimating metrics of hydraulic vulnerability, such as leaf water potential (Ψ_{leaf}) at 50% loss of hydraulic conductance (P_{50}). For example, a species' value of $\mathrm{RWC@PLRC}_{10}$ of 75% signifies that a leaf bench dried to an RWC of 75% (of $\mathrm{SWC}_{\mathrm{orig}}$) and after 8 hr of rehydration recovered to RWC of 90% (of $\mathrm{SWC}_{\mathrm{orig}}$); that is, it lost 10% of its rehydration capacity. Notably, we did not calculate RWC at PLRC_{50} for *Quercus agrifolia* and *Quercus berberidifolia*, which never reached this level of dysfunction even under the most extreme dehydration treatments were imposed (see Section 3). Secondly, we calculated the PLRC at key levels of leaf dehydration, that is, at (a) PLRC at Ψ_{leaf} = −2 MPa (used as an arbitrary level of moderate–severe dehydration, below turgor loss point for many species), (b) at RWC = 70% (corresponding to significant loss of leaf viability; Oppenheimer & Leshem, 1966), (c) at Ψ_{leaf} corresponding to 50% and 80% loss of stomatal conductance, and (d) turgor loss point. The Ψ_{leaf} values corresponding to 50% and 80% stomatal closure were determined for the same individual plants from a subset of 14 of the study species in shoot drying experiments in which leaves were measured for stomatal conductance (using a porometer; AP-4, Delta-T Devices Ltd, Cambridge, UK) and Ψ_{leaf} (using a pressure chamber) during progressive dehydration under high irradiance (Guyot, Scoffoni, & Sack, 2012; Henry et al., unpublished). Because the plant material required for stomatal closure experiments was considerably greater than for this rehydration study, four species in this study (*Cleome isomeris*, *Quercus engelmannii*, *Rhus integrifolia*, and *Salvia leucophylla*) were not included in stomatal closure experiments.

The Ψ_{leaf} corresponding to the RWC for given PLRC was determined from the pressure–volume parameters of each species (Sack, John, & Buckley, 2017).

2.4 | Pressure–volume curves

We extracted species drought tolerance parameters from pressure–volume curves determined using the bench-dry method (Sack & Pasquet-Kok, 2011). For six to nine plants per species, five to six leaves were measured for leaf water potential and mass during dehydration. From the plotted curves; we determined osmotic potential at full turgor (π_o) and water potential at turgor loss point (π_{tlp}), RWC at turgor loss point ($\mathrm{RWC}_{\mathrm{tlp}}$), modulus of elasticity (ε), and capacitance at full turgor (C_{ft}). For two species, *C. spinosus* and *Encelia californica*, pressure–volume curves were not determined given difficulty establishing equilibration and petiole fragility.

To test whether leaves tended to oversaturate during initial rehydration, that is, whether RWC increased above 1, we assessed the data from pressure–volume curves. We quantified the RWC of all the leaves when they were maximally hydrated at the beginning of pressure–volume curve determination, with initial leaf water potential greater than −0.2 MPa. To estimate this RWC for each leaf, we used the initial SWC derived from the pressure–volume curve as the *y*-intercept of the leaf water content versus leaf water potential for the points above turgor loss point (Sack & Pasquet-Kok, 2011).

2.5 | Minimum cuticular conductance

We measured minimum epidermal conductance (g_{min}) for six to 20 leaves for each species. After rehydrating individual leaves for >8 hr, we measured saturated leaf mass (±0.01 mg; MS205DU; Mettler Toledo, OH) and area (flatbed scanner; ImageJ software Version 1.42q; National Institutes of Health). Leaves were suspended by their petioles over a fan for 30 min to 2 hr to force stomatal closure and then weighed at 15-min intervals for at least 2 hr, after which leaf area was measured again. We collected ambient temperature and relative humidity at 30-s intervals using a weather station (HOBO Micro Station with Smart Sensors; Onset, Bourne, MA, USA). After measurements, leaves were oven dried for 3 days at 70°C and weighed for dry mass, and g_{min} was calculated from the minimum epidermal transpiration rate divided by mole fraction vapour pressure deficit and by the twice the mean projected leaf area (Sack & Scoffoni, 2011).

2.6 | Ecosystem range climate variables

We calculated species native range mean annual temperature (MAT), mean annual precipitation (MAP), aridity index (AI), and potential evapotranspiration (PET) on the basis of occurrence data extracted from the Global Biodiversity Information Facility (GBIF.org (4th August 2015) see Table S1 for Occurrence Download DOIs http://www.gbif.org). We projected points on a map using ArcMAP (http://www.esri.com/software/arcgis) and deleted false occurrences (i.e., points in the ocean), collections that appeared outside of the natural geographic range, and coordinates that matched to political centroids (i.e., coordinates that would refer to the centre of countries or states, used when herbarium data with unknown exact locations are entered in GBIF after Edwards et al., 2015). Using these cleaned data, we obtained MAT and MAP for each locality from WorldClim–Global Climate Data website (http://www.worldclim.org/; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) using a 2.5 arc-minute resolution from the generic grids and the R raster package (Hijmans & van Etten, 2015). We obtained PET and AI from the CGIAR-CSI database, also calculated using WorldClim data.

2.7 | Survey of literature for variation in rehydration capacity

To broaden the analysis of the loss of rehydration capacity as a drought tolerance trait, we examined the patterns in PLRC across native ecosystems, growth forms, and leaf habit on the basis of the published literature. We conducted searches using Web of Science (using as keywords "rehydration capacity," "sublethal water deficit," "leaf mortality," "lethal relative water content," "leaf dehydration threshold," "leaf death," etc.) to compile datasets featuring measurements of PLRC and recorded the RWC at $PLRC_{10}$, $PLRC_{25}$, and $PLRC_{50}$, the native ecosystem, leaf habit, and growth form (Table S7).

2.8 | Statistics

To test for an effect of rehydration time, we applied a repeated-measures analysis of variance (ANOVA), that is, a nested general linear model nesting leaf within species as a random effect, with time as a fixed effect (Minitab Release 15; Minitab, State College, Pennsylvania, USA). This nested model allowed assessment for variation between time points both within and across species. To test for oversaturation during rehydration, we calculated the across species mean RWC for the fully hydrated leaves and applied a *t* test to determine whether the value was greater than 1 (Minitab Release 15).

For the California species, we tested for trait differences across leaf habit types (evergreen vs. deciduous) and ecosystems (chaparral vs. woodland) with a one-way multivariate analysis of variance (MANOVA) using the aov function in R (Chambers, Freeny, & Heiberger, 1992). We grouped traits into four categories: levels of RWC at given PLRC, PLRC at key stages of leaf dehydration, other leaf water relations traits (i.e., stomatal response and pressure–volume parameters), and leaf structural and compositional traits (e.g., LMA; see Table 1). While the analyses of leaf habit and growth forms included all species from desert, chaparral, and woodland, we tested ecosystem differences only between woodland and chaparral and excluded desert species due to low species representation.

We also tested for differences within our database compiled from the published literature as an expanded test of the generality of our findings. We applied one-way MANOVAs with a post hoc Tukey honestly significant difference (HSD) test using the aov (Chambers et al., 1992) and Tukey HSD (R. G. Miller, 1981; Yandell, 1997) functions in R to test for statistical differences between leaf habits (evergreen or deciduous), or among growth forms (tree, shrub, or grass), or ecosystems (tropical forest, temperate forest, Mediterranean woodland, Mediterranean chaparral, or grassland).

All data were log-transformed before ANOVAs and t tests to improve normality and reduce heteroscedasticity (Sokal & Rohlf, 1981).

We analysed ahistorical trait correlations and evolutionary correlations among variables to assess both potential physically based mechanisms (e.g., the correlation of PLRC with elastic modulus, which might directly contribute to leaf cell dehydration tolerance) and evolutionary shifts in traits with climate. We tested for ahistorical correlations using the lm function in R (Chambers, 1992; Wilkinson & Rogers, 1973) on both untransformed and log-transformed data to model respectively approximately linear or non-linear (approximately power law) relationships (R statistical software; Sokal & Rohlf, 1981).

We tested phylogenetically independent contrasts (PICs) using a Brownian motion model of trait evolution to account for phylogeny in trait relationships. We used Phylomatic (Stoltzfus et al., 2013) to build a time-calibrated phylogeny of our species list (based on Zanne et al., 2014) and collapsed singleton nodes using the R package ape (Paradis, Claude, & Strimmer, 2004). Low phylogenetic resolution at relatively recent divergences (e.g., splits between *Quercus* species) produced unreliable contrast statistics due to large trait differences over short branch lengths, and thus, we collapsed the tree to genus level to produce a more rigorous test for phylogenetic signal. We calculated PICs using the phytools package (Felsenstein, 1985; Revell, 2012) and tested for correlations across PICs using the lm function in R (Chambers, 1992; Wilkinson & Rogers, 1973) forced through the origin (Garland, Harvey, & Ives, 1992) on log-transformed data.

3 | RESULTS

3.1 | Timing of recovery of leaf water content after dehydration and tests for oversaturation

We tested the timing of leaf rehydration on a subset of eight species selected to span the range of drought sensitivity (π_{tlp} = −1.8 to −3.0 MPa), leaf habits, and native ecosystems (indicated by asterisks in Table 2). On average across species, leaves achieved their maximum rehydration within 8 hr after petioles were submerged; RWC differed between 0 and 8 hr of rehydration, but not between 8 and 12 hr, or 12 and 24 hr (Figure 1; $P < 0.001$; repeated-measures ANOVAs). Our additional tests of pressure–volume curve data did not support significant oversaturation. Our analysis showed that across the leaves of 14 species that could be tested for oversaturation (i.e., those for which maximally hydrated leaves in the curve had leaf water potential greater than − 0.2 MPa), the mean RWC

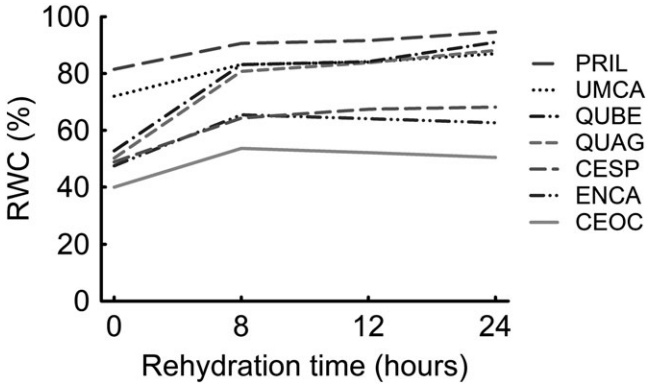

FIGURE 1 Recovery of leaf water content for dehydrated leaves after different recovery (rehydration) times for seven species. Lines show species' mean relative water contents (RWC) across eight to 17 leaves through time with initial values ranging from 9% to 97%. On average, leaves recovered water status within 8 hr of rehydration. One species, *Salvia leucophylla*, was not included as its water status was assessed in terms of total leaf mass, rather than relative water content (see Table S2). See Table 1 for species names

of maximally hydrated leaves was 1.01 and was not significantly greater than 1 (*t* test; $P = 0.28$).

3.2 | Loss of rehydration capacity in dehydrated leaves

As expected from previous studies, after a given level of dehydration, species typically showed a linear loss of rehydration capacity with ongoing dehydration. When plotting the PLRC against the dehydrated RWC, some species showed loss of rehydration capacity immediately with the onset of dehydration (*Alnus rhombifolia*, *Betula occidentalis*, *Cercis occidentalis*, *Encelia farinosa*, *Fraxinus dipetala*, *Malosma laurina*, *Platanus racemosa*, *Prunus ilicifolia*, and *Umbellularia californica*), whereas others exhibited an apparent plateau above RWC of 90%, before declining linearly (Figure 2; $R^2 = 0.18–0.96$, $P < 0.001–0.05$). We fitted lines to data points below RWC of 90% dehydration and determined RWC at $PLRC_{10}$, $PLRC_{25}$, and $PLRC_{50}$. For 16 of 18 species, sufficiently strong dehydration resulted in PLRC > 50%. However, for *Q. agrifolia* and *Q. berberidifolia*, leaves subjected to dehydration even to RWC < 10% were able to recover to 58–60% of original SWC (Figure 2).

3.3 | Species variation in the capacity to rehydrate after dehydration—Water status thresholds

We found $PLRC_{10}$ to occur on average at 65.9 ± 4.2%, consistent with expectations from previous work showing that metabolic damage begins at approximately RWC of 70% (Oppenheimer & Leshem, 1966). Yet species varied strongly in their thresholds for loss of rehydration capacity (Table 2). The RWC at $PLRC_{10}$ varied from 30.5% for *C. spinosus* to 85.5% for *B. occidentalis*, the RWC at $PLRC_{25}$ varied from 24.1% for *C. spinosus* to 70.6% for *E. farinosa*, and the RWC at $PLRC_{50}$ varied from 3.1% for *Q. agrifolia* to 57.2% for *E. farinosa*. Notably, even with very strong dehydration to below 10% RWC, *Q. agrifolia* and *Q. berberidifolia* retained strong rehydration capacity, such that $PLRC_{50}$ was not measurable without extrapolating past the data. Corresponding thresholds for PLRC in terms of leaf water potential (Ψ_{leaf}) also varied strongly across species. The Ψ_{leaf} at $PLRC_{10}$ varied from −0.92 MPa for *A. rhombifolia* to −4.5 MPa for *C. betuloides*, the Ψ_{leaf} at $PLRC_{25}$ from −1.7 MPa for *A. rhombifolia* to −6.5 MPa for *Q. berberidifolia*, and the Ψ_{leaf} at $PLRC_{50}$ was less than −2.3 MPa for the 13 species for which it could be quantified, that is, excluding *B. occidentalis*, *Q. agrifolia*, and *Q. berberidifolia* (Table S1).

Species also varied strongly in their PLRC at key stages of dehydration. The PLRC at −2 MPa varied from 0% for *C. betuloides*, *M. laurina*, *Q. agrifolia*, *Q. berberidifolia*, and *R. integrifolia* (i.e., no loss

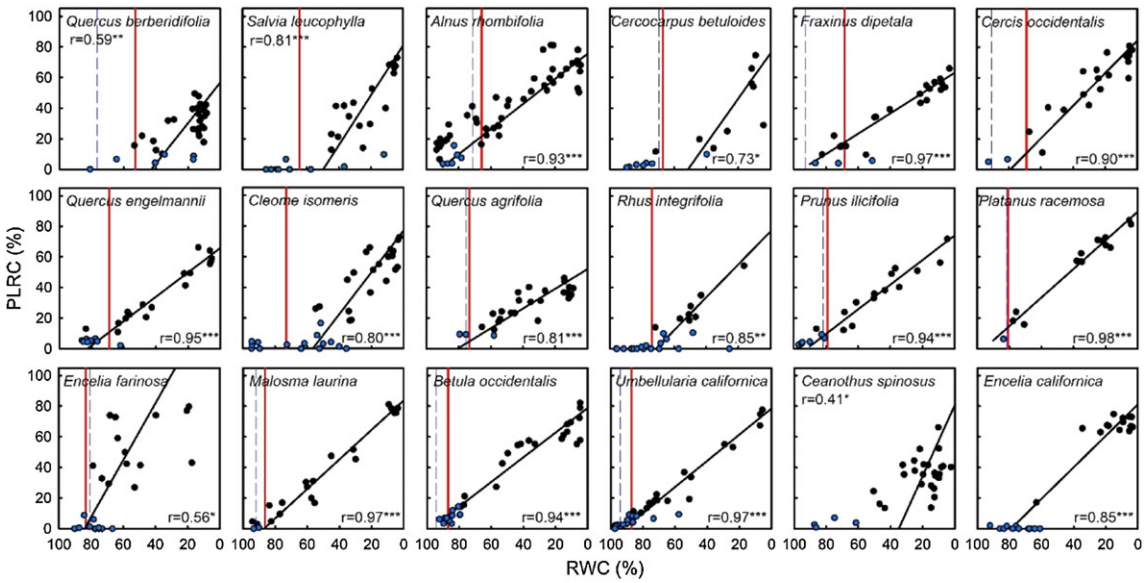

FIGURE 2 Relationships between the percentage loss of leaf rehydration capacity (PLRC) and relative water content (RWC) at dehydration for 18 species native to Southern California. Also shown, when data were available, is the RWC at which turgor is lost (RWC_{tlp}; solid red vertical lines) and 50% of stomatal conductance is lost (RWC_{SC50}; blue dashed vertical lines). Trend lines were fitted with ordinary least squares to points below $PLRC_{10}$ (black points). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

of rehydration capacity after dehydration to −2 MPa) to 39.1% in *A. rhombifolia*. The PLRC at 50% stomatal closure varied from 0% for *C. betuloides*, *C. occidentalis*, *M. laurina*, *Q. berberidifolia*, and *U. californica* to 17.0% for *A. rhombifolia*. The PLRC at 80% stomatal closure varied from 0% for *Q. berberidifolia* to 54.0% for *P. ilicifolia*. Similarly, PLRC at turgor loss point varied from 0% for five species that fully rehydrated after dehydrating to turgor loss point (*C. isomeris*, *C. betuloides*, *Q. berberidifolia*, *R. integrifolia*, and *S. leucophylla*) to 21.7% for *A. rhombifolia*. Finally, the PLRC at 70% RWC varied from 0% for *C. isomeris*, *C. betuloides*, *C. spinosus*, *Q. berberidifolia*, and *S. leucophylla* to 26.3% for *E. farinosa*.

Leaf dehydration tolerance varied on average across native ecosystems. On average, species native to woodlands had leaves more sensitive to dehydration than species of the chaparral, with mean values 20–22% higher for RWC at $PLRC_{10}$ and at $PLRC_{25}$ ($P = 0.03$; $n = 7$ and 8; one-way MANOVA; Table 3). Values of PLRC at turgor loss point and at leaf water potential of −2 MPa were on average 10% higher for species native to woodlands than for those of chaparral ($P = 0.04$).

Leaf dehydration tolerance also varied according to species' leaf habit. Deciduous species had leaves more sensitive to desiccation than had evergreen species, with on average 14–19% higher values for RWC at $PLRC_{10}$ and at $PLRC_{25}$ and at $PLRC_{50}$ ($P = 0.03$–0.009; $n = 7$ and 11). PLRC at turgor loss point and at −2 MPa was on average 9% and 15% higher, respectively, for deciduous than for evergreen species ($P = 0.2$ and $P = 0.002$, respectively).

Tree and shrub species did not differ statistically in any indices of leaf dehydration tolerance ($P = 0.11$–0.89; $n = 10$ and 8).

3.4 | Variation in other structural and drought tolerance traits

The study species varied strongly in other drought tolerance traits (Table S4). Species varied 13-fold in g_{min}, from 1.19 ± 0.1 mmol·m^{-2}·s^{-1} for *U. californica* to 16.6 ± 0.8 mmol·m^{-2}·s^{-1} for *C. isomeris*. Species varied in π_o from −0.80 ± 0.05 MPa for *S. leucophylla* to −2.3 ± 0.04 MPa for *M. laurina*; in π_{tlp} from −1.2 ± 0.02 for *S. leucophylla* to −3.0 ± 0.1 for *Q. agrifolia*; and in RWC_{tlp} from 58.5 ± 3.5% for *Q. berberidifolia* to 87.1 ± 9.3% for *U. californica*. Capacitance at full turgor (C_{ft}) and modulus of elasticity varied from 0.05 ± 0.005 MPa^{-1} and 18.0 ± 1.9 MPa, respectively, for *M. laurina* to 0.33 ± 0.03 MPa^{-1} and 2.1 ± 0.5 MPa, respectively, for *S. leucophylla*.

The study species varied strongly in structure, with twofold variation in LT, 70-fold in leaf area, threefold in LD, nearly fourfold in LMA, and over sixfold in leaf saturation water content (Table S1).

On average, species native to chaparral had −0.4 MPa lower π_{tlp} ($P = 0.001$) and 23% lower SWC ($P < 0.001$) than had woodland species. Similarly, evergreen species were 0.5 MPa more negative in π_{tlp} ($P = 0.002$) and 44% lower in SWC than were deciduous species ($P = 0.04$; Table 3).

3.5 | Loss of rehydration capacity within the sequence of drought responses

We observed a consistent average sequence of drought responses across species, with loss of substantial rehydration capacity ($PLRC_{10}$) occurring before complete stomatal closure (Figure 3). As leaves dehydrated, 50% of stomatal conductance was lost at RWC of 83.4 ± 2.6% and Ψ_{leaf} of −1.4 ± 0.2 MPa on average across species; turgor was lost on average at RWC of 74.1 ± 2.2% and Ψ_{leaf} of −2.1 ± 0.1 MPa; and 80% of stomatal conductance was lost at RWC of 67.3 ± 5.6% and Ψ_{leaf} of −2.4 ± 0.4 MPa. $PLRC_{10}$ occurred on average at RWC of 65.9 ± 4.2% and Ψ_{leaf} of −2.4 ± 0.3 MPa, $PLRC_{25}$ at 51.0 ± 4.2% and −3.3 ± 0.4 MPa, and $PLRC_{50}$ at 26.1 ± 3.1% and −5.5 ± 1.3 MPa.

While two species (17% of species) reached $PLRC_{10}$ before RWC_{SC50}, on average across all species, $PLRC_{10}$ occurred after RWC_{SC50} (paired *t* test on log-transformed data; $P = 0.03$; Tables S3 and S4). Turgor was lost before $PLRC_{10}$ ($P = 0.05$), but at statistically

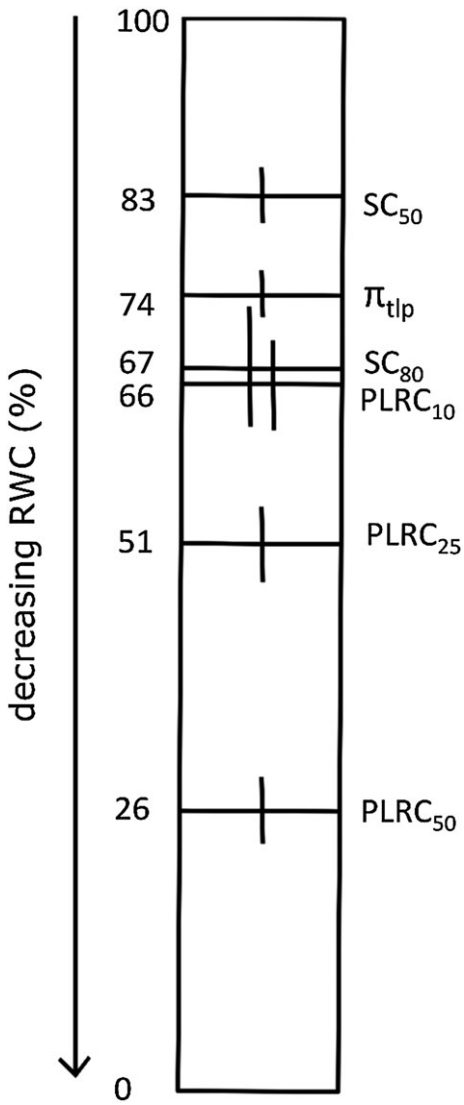

FIGURE 3 Theoretical sequence of responses of stomatal closure, wilting, and loss of rehydration capacity with leaf dehydration, in terms of leaf relative water content. As relative water content is lost (from 100% theoretical maximum saturation to 0%), first 50% of stomatal conductance is lost (SC_{50}), then leaf turgor is lost (π_{tlp}), 80% of stomata conductance is lost (SC_{80}), 10% of the capacity to rehydrate is lost ($PLRC_{10}$), 25% of the capacity to rehydrate ($PLRC_{25}$) is lost, and finally 50% of the capacity to rehydrate is lost ($PLRC_{50}$). This same pattern holds as leaf bulk water potential declines. See Table S4 for statistical results

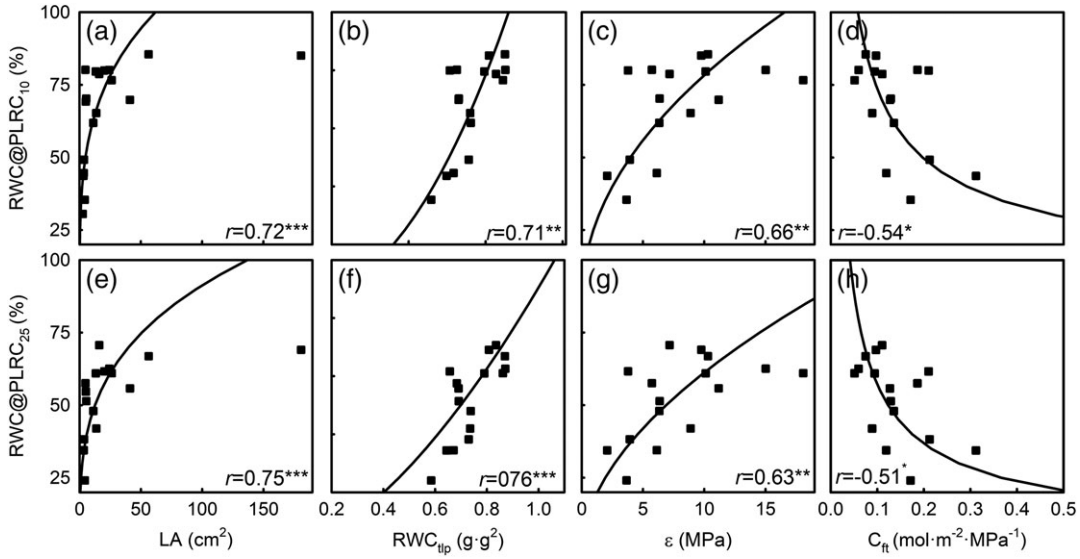

FIGURE 4 Relationships between relative water content at 10% (RWC@PLRC$_{10}$; a–d) and 25% (RWC@PLRC$_{25}$; e–h) loss of rehydration capacity and leaf functional traits including leaf area (LA), relative water content at turgor loss point (RWC$_{tlp}$), modulus of elasticity (ε), and leaf capacitance at full turgor (C_{ft}). Lines were fitted with standard major axis assuming linear or power law relationship according to which showed stronger fit. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All significant relationships remained significant after correction with phylogenetically independent contrasts ($P \leq 0.001$–0.03; Table S6)

similar RWC as 80% loss of stomatal conductance ($P = 0.19$). Across species, the mean PLRC@SC$_{50}$ was 4.4 ± 1.7%, and PLRC@π_{tlp} was 6.6 ± 1.8%. PLRC$_{25}$ occurred at statistically similar RWC to 80% loss of stomatal conductance ($P = 0.21$), and PLRC$_{50}$ occurred significantly beyond that point ($P = 0.003$).

3.6 | Correlations across species among traits and climate

The RWCs at PLRC$_{10}$, PLRC$_{25}$, and PLRC$_{50}$ were intercorrelated across species (r = 0.69–0.98, $P < 0.001$; Table S5). Further, the RWC@PLRC$_{10}$ and RWC@PLRC$_{25}$ were strongly correlated with the RWC at turgor loss point (Figure 4b and 4f; r = 0.71–0.75, $P < 0.001$) and to a lesser extent, ε (Figure 4c and 4g; r = 0.63–0.66, P = 0.006–0.009). Similarly, Ψ@PLRC$_{10}$, Ψ@PLRC$_{25}$, and Ψ@PLRC$_{50}$ were intercorrelated (r = 0.90–0.97, $P < 0.001$) and correlated with π_{tlp} (Figure 5e and 5j; r = 0.76, $P_p < 0.001$).

Rehydration capacity was also correlated with leaf dimensions and functional traits. The RWCs at PLRC$_{10}$ and PLRC$_{25}$ were lower for species with smaller leaves (Figure 4a and 4e; r = 0.72–0.73, $P < 0.001$), the Ψ_{leaf} at PLRC$_{10}$ and PLRC$_{25}$ were related to LMA, LD, and SWC (Figure 5a, c, d, f, h, and i; r = −0.68 to 0.88, $P < 0.001$–0.003), and the Ψ_{leaf} at PLRC$_{10}$ was related to LT (Figure 5b and 5h; r = 0.51, P = 0.04), such that species with thicker

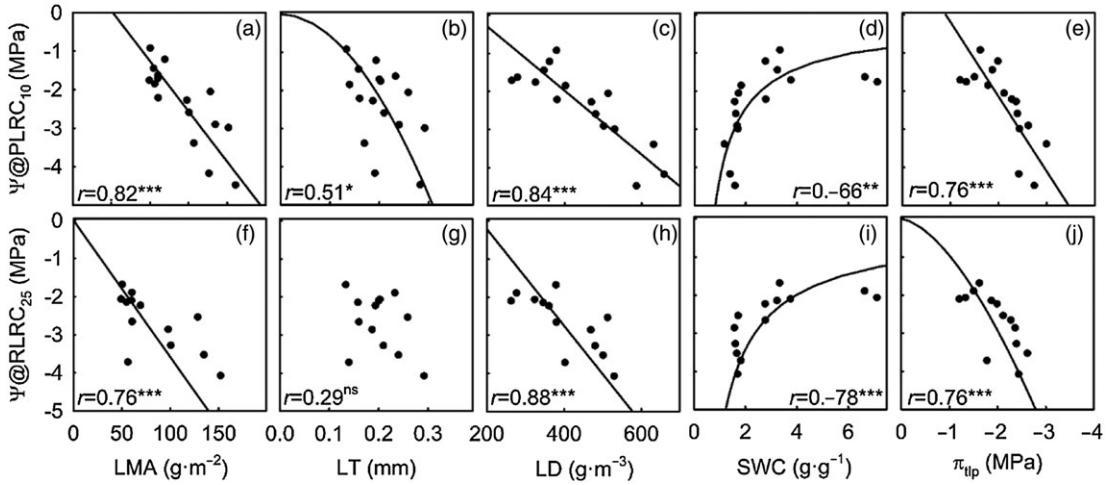

FIGURE 5 Relationships between leaf water potential at 10% (Ψ@PLRC$_{10}$; a–e) and 25% (Ψ@PLRC$_{25}$; f–j) loss of rehydration capacity and leaf functional traits including leaf mass per area (LMA), leaf thickness (LT), leaf density (LD), saturated water content (SWC), and bulk leaf water potential at turgor loss point (π_{tlp}). Lines were fitted with standard major axis assuming linear or power law relationship according to which showed stronger fit. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All significant relationships remained significant after correction with phylogenetically independent contrasts (P = 0.002–0.01; Table S6)

and denser leaves with higher LMA and lower SWC had greater rehydration capacity. Indeed, the PLRC at −2 MPa was negatively correlated with LMA, LD, and π_{tlp} (r = −0.78 to −0.85) and positively correlated with SWC (Figure 6g–j; r = 0.73, P < 0.001–0.001), and the PLRC at turgor loss point was negatively correlated with LT (Figure 6a; r = −0.60, P = 0.045).

Across species, leaf rehydration capacity was not significantly related to climatic variables in an ahistoric correlation analysis (P = 0.16–0.99). However, the evolutionary correlation analysis resolved an association with adaptation to greater aridity; PLRC@π_{tlp} was positively related to MAP (r = 0.57, P = 0.03), Ψ@PLRC$_{25}$ was negatively related to PET (r = −0.56, P = 0.04), and RWC$_{SC50}$ was negatively related to both PET and MAT (r = −0.74, P = 0.009, and r = −0.74, P = 0.01).

3.7 | Comparison of leaf rehydration capacity for Southern California species with previously compiled data from global ecosystems

Our search for all available data in the literature for RWC@PLRC$_{10}$, RWC@PLRC$_{25}$, and RWC@PLRC$_{50}$ resulted in a novel global database for 89 species native to montane and lowland wet tropical forests, temperate forests, Mediterranean ecosystems, and grasslands from 17 publications (Table S7; Oppenheimer, 1963; Rychnovská-Soudková, 1963; Oppenheimer & Leshem, 1966; Rychnovska, 1967; Weinberger et al., 1972; Buckley et al., 1980; Florineth, 1980; Peace & Macdonald, 1981; Rambousková, 1981; Hinckley et al., 1983; Goldstein et al., 1985; Kapos & Tanner, 1985; Park, 1989; Alexandre, 1991; Burghardt & Riederer, 2003; Burghardt et al., 2008). The mean RWC@PLRC$_{10}$ across the California species tested in this study (65.9 ± 4.2%) was similar to that across our global database (61.4 ± 1.8%, P = 0.31; unpaired t test).

Ecosystems differed in mean RWC@PLRC$_{10}$: Species from wet tropical forests and Mediterranean woodlands showed relatively low dehydration tolerance, with progressively lower values for species of Mediterranean chaparral, temperate forest, and grasslands (Figure 7). Grasses had significantly lower RWC@PLRC$_{10}$ than had the leaves of trees or shrubs (P = 0.001 and P < 0.001; Table S8). In this database analysis, there was no significant difference between evergreen leaves and deciduous leaves across all ecosystems (P = 0.79), even if nested within ecosystems (P = 0.57) or excluding grass species (P = 0.67 and P = 0.18, respectively). Grasslands also differed from all other ecosystems in their lower RWC@PLRC$_{10}$ and RWC@PLRC$_{25}$ on average (P < 0.001), but not in their RWC@PLRC$_{50}$ (P = 0.93–0.98).

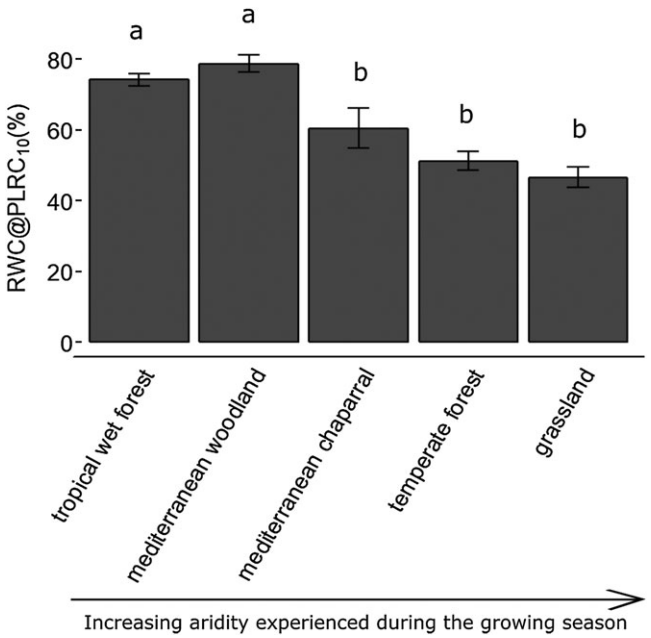

FIGURE 7 Means for species of given ecosystems for relative water content at 10% loss of rehydration capacity (RWC@PLRC$_{10}$). Different letters indicate significant differences (Tukey's honestly significant difference tests). Means were calculated for species featured in multiple studies (Table S7)

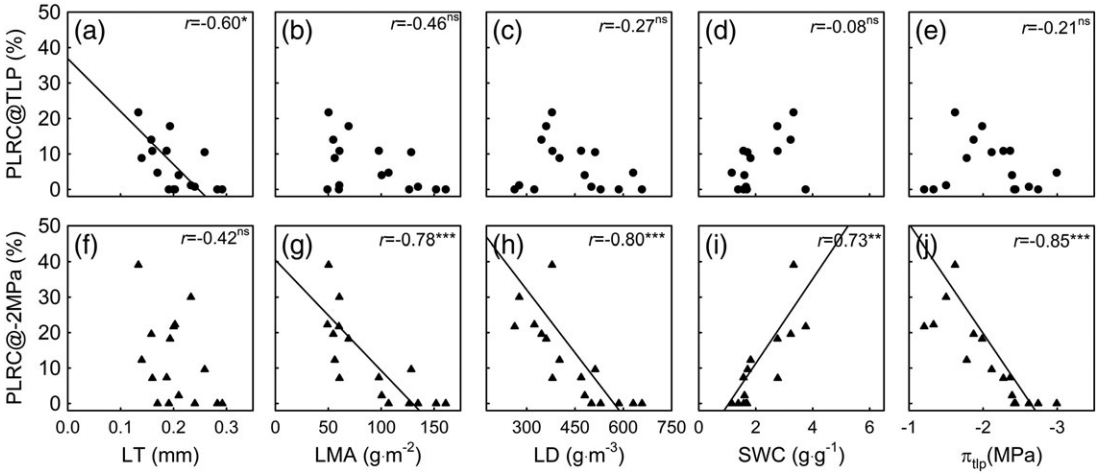

FIGURE 6 Relationships between the percent loss of rehydration capacity at turgor loss point (PLRC@π_{tlp}; a–e) and −2 MPa (PLRC@−2 MPa; f–j) and key leaf functional traits including leaf thickness (LT), leaf mass per area (LMA), leaf density (LD), saturated water content (SWC), and bulk leaf water potential at turgor loss point (π_{tlp}). Lines were fitted with standard major axis. *P < 0.05, **P < 0.01, ***P < 0.001. Most significant relationships remained significant after correction with phylogenetically independent contrasts; however, the relationship between PLRC@π_{tlp} and leaf thickness was not significant after phylogenetic correction (P = 0.22–0.97; Table S6)

4 | DISCUSSION

We updated a classical method (Oppenheimer, 1963) to quantify leaf viability loss and dehydration tolerance across species diverse in physiology and natural history. Our study demonstrated a wide range of leaf rehydration capacity across species within and across California ecosystems. For many species, substantial loss of rehydration capacity occurred at milder leaf water potentials than at those corresponding to complete stomatal closure. Thus, leaves may be more sensitive to permanent shrinkage than previously believed and, once having experienced a drought to stomatal closure, may not recover to operate at their "optimum" water status. Leaf dehydration tolerance as assessed by rehydration capacity was linked with leaf habit, leaf structure, and other drought tolerance metrics and environmental variables for California species, and in our global analysis of published data.

4.1 | Recovery of leaf water content after dehydration—Rehydration time

We firstly tested the timing required for leaf rehydration. On average, leaves rehydrated fully after 8 hr, validating the use of overnight rehydration as sufficient, even for leaves dehydrated to levels at which the petiole and vein xylem would be partially embolized (Garnier et al., 2001; Brodribb, Carriqui, Delzon, & Lucani, 2017; Scoffoni et al., 2017; see further discussion below). We saw little impact of leaf oversaturation after rehydration that would have influenced our estimation of PLRC. Oversaturation during rehydration has been previously reported for a range of species, especially grasses (Arndt et al., 2015; Kubiske & Abrams, 1991; Rychnovska, 1967), and may have occurred infrequently in our leaves due to our rehydration protocols and/or initial drought hardening treatment.

4.2 | Recovery of leaf water content after dehydration—Relationship to degree of dehydration

We found a general linear relationship between PLRC and RWC at dehydration. Whereas most studies found similar trends (Alexandre, 1991; Buckley et al., 1980; Burghardt et al., 2008; Burghardt & Riederer, 2003; Goldstein et al., 1985; Hinckley et al., 1983; Kapos & Tanner, 1985; Oppenheimer, 1963; Park, 1989; Peace & Macdonald, 1981; Weinberger et al., 1972), some previous work found water content recovery to drop exponentially with leaf dehydration (Florineth, 1980; Rambousková, 1981; Rychnovska, 1967; Sommerville & Read, 2008). The linear relationship would indicate that progressive dehydration leads to damage or death of a proportional number of cells such that they cannot rehydrate to their maximum capacity (Kramer & Boyer, 1995; Oppenheimer & Leshem, 1966). Cells are expected to be damaged or killed by shifts in ion concentrations outside the range that can be tolerated, damage to the cytoskeleton and/or to cell and/or organelle membranes and their embedded transporters and other proteins, and/or to accumulated free radical damage (Foyer & Noctor, 2005; G. A. D. Miller, Suzuki, Ciftci-Yilmaz, & Mittler, 2010; Mittler, 2002; Sharma, Jha, Shanker, & Pessarakli, 2012). Notably, leaf rehydration capacity is a bulk parameter with future work needed to resolve the specific cells affected and the changes undergone, that is, irreversible volume shrinkage in certain cells due to membrane dysfunction and/or loss of metabolic activity or even to death of cells. Using viability staining in dehydration experiments for *Nerium oleander*, Oppenheimer found mesophyll cells to be most sensitive to loss of viability, indicated by failure to take up neutral red stain, followed by those cells closest to the vascular bundle (likely bundle sheath, though not specified), with the thicker walled epidermal cells far more resistant than are other cell types (Oppenheimer & Leshem, 1966). However, the generality of those results remains untested. We have also assumed that PLRC is not strongly related directly to accumulation of xylem embolism. Although severely dehydrated leaves would indeed have embolism in their vein xylem, especially in the midrib, which, on average across species, contains a substantial minority of leaf water (ranging from 5% to 42% across 14 measured dicotyledonous species; 22 ± 3% mean ± standard error; unpublished data), we assume that during rehydration any embolized vessels at the cut petiole end would be refilled through capillary action and that embolized conduits would refill over the course of hours of rehydration due to surface tension-driven bubble dissolution with vein water at atmospheric pressure (Yang & Tyree, 1992). These assumptions need further validation.

Despite the typical linear response of PLRC to RWC, our study identified greater diversity in leaf dehydration tolerance than expected on the basis of the previous literature. For example, while sublethal water deficit has been often defined as the RWC@$PLRC_{10}$ on the basis that this is the point at which notable damage begins (Buckley et al., 1980; Burghardt et al., 2008; Florineth, 1980; Goldstein et al., 1985; Kapos & Tanner, 1985; Oppenheimer & Leshem, 1966; Weinberger et al., 1972), for six of our 18 species (*A. rhombifolia*, *B. occidentalis*, *M. laurina*, *P. racemosa*, *P. ilicifolia*, and *U. californica*), a linear decline of PLRC began above RWC of 90%. By contrast, leaves of three of our study species (*C. spinosus*, *Q. agrifolia*, and *Q. berberidifolia*) recovered from extreme water deficits (<10% RWC) to 48–66% of their initial pre-dehydration SWC. These species are evergreens native to chaparral, adapted to maintaining leaves through severe drought events (Venturas et al., 2016). In this way, they show a degree of "resurrection" type behaviour, such as that found in species that can recover from virtually complete dehydration (>300 pteridophytes and angiosperms [Rascio & Rocca, 2005];e.g., *Boea hygrometrica* [Xiao et al., 2015], *Craterostigma wilmsii* [Cooper & Farrant, 2002], *Haberlea rhodopensis* [Moyankova et al., 2014], and *Selaginella lepidophylla* [Eickmeier, 1979]). Further study is needed in areas that experience extreme drought cycles, especially within the growing period of the year, to establish the metabolic and functional viability of rehydrated leaves.

4.3 | Loss of leaf rehydration capacity in the sequence of drought response

On average across species, the RWC corresponding to $PLRC_{10}$ was 65.9 ± 4.2%, consistent with previous work (Table S7) and Oppenheimer's value of 70% (Oppenheimer & Leshem, 1966). Notably, extreme dehydration was required for $PLRC_{50}$, well beyond the range of conditions that leaves experience outside of the most

extreme drought events. Whereas in other systems "lethal doses" are often quantified as 50% loss of viability, for leaves, $PLRC_{10}$ appears to better represent damage and resolve species differences.

On average across our species, stomata began to close at high RWC, followed by loss of leaf turgor, 80% stomatal closure, and $PLRC_{10}$ (Figure 3). Many species suffered some damage by turgor loss point and by 50% stomatal closure with PLRC values of 0–22% (average across species of 7%) and 0–17% (average across species of 4%), respectively. Our finding of the severity of turgor loss point is consistent with the results of a recent meta-analysis indicating that plants rarely reach turgor loss point during typical seasonal droughts (Bartlett, Klein, Jansen, Choat, & Sack, 2016). Our findings also challenge the notion that stomata close completely early enough to protect tissues from significant damage and instead indicate that many species avoid stomatal closure even at the cost of leaf viability, in terms of PLRC. Indeed, the average $PLRC_{10}$ across species (−2.4 MPa) occurred before the threshold that has been suggested for stomatal closure to avoid rapid loss of viability and ultimately leaf death via hydraulic failure (−4 MPa; Martin-StPaul, Delzon, & Cochard, 2017). Burghardt and Riederer (2003) found that for three deciduous and two evergreen plant species, complete stomatal closure occurred above the turgor loss point (RWC of 80–90%) and well before water deficits resulting in $PLRC_{10}$ (RWC at $PLRC_{10}$ = roughly 50% across their species), in contrast with our findings. Notably, the species in that study were far less drought tolerant than our Southern California species and thus may exhibit different stomatal behaviours. Further, the difference may arise from the estimation of water status at stomatal closure, which was alternatively measured in the dark (Burghardt & Riederer, 2003) or for illuminated leaves (this study); several studies have shown that stomata remain substantially open for illuminated leaves even to bulk leaf turgor loss (Guyot et al., 2012; Hernandez-Santana, Rodriguez-Dominguez, Fernández, & Diaz-Espejo, 2016). Although our findings for loss of cell integrity or viability before stomatal closure necessitate reconsideration of some common ideas of stomatal protection, they are consistent with those of several recent studies that showed substantial reductions in leaf hydraulic conductance (K_{leaf}) before stomata closed by 50% (Bartlett et al., 2016; Brodribb et al., 2003; Johnson, Woodruff, McCulloh, & Meinzer, 2009). The mechanism for K_{leaf} decline with dehydration remains under debate with recent research attributing this decline to xylem embolism and decline in conductance in the outside-xylem pathways (Brodribb et al., 2017; Scoffoni & Sack, 2017; Skelton, Brodribb, & Choat, 2017; Trifiló, Raimondo, Savi, Lo Gullo, & Nardini, 2016). As substantial leaf vein embolism has only been directly observed below turgor loss point (Brodribb et al., 2017; Scoffoni et al., 2017) and well after stomatal closure (Hochberg et al., 2017; Skelton, Brodribb, & Choat, 2017), the initial hydraulic decline at high leaf water potentials has been attributed to the hydraulic pathway outside the leaf xylem (Scoffoni et al., 2017). Notably, $PLRC_{10}$ also typically occurs at milder water potentials than that at which vein xylem embolism has been directly observed. Maintaining open stomata despite losses of K_{leaf}, irreversible shrinkage, and/or possible death to some mesophyll cells may enable a sustained photosynthetic rate at the onset of drought, which may be important to mitigate energy costs associated with active drought responses (e.g., osmotic adjustment).

4.4 | Variation in species recovery in water status with ecosystem and leaf habit

The relationship between sensitivity to loss of leaf rehydration capacity and environment was supported by the greater sensitivity of Southern California species native to woodlands than of those native to chaparral, and by the evolutionary correlations of PLRC with climatic variables reflecting aridity in their native ranges. Notably, the two species of driest climates were desert species, yet these species generally showed less dehydration tolerance than did those native to chaparral, consistent with previous work showing that the leaves of many desert species are adapted to operate when water is relatively abundant (Grubb, 1998; Maximov, 1929).

Species with evergreen leaves showed less sensitivity to loss of leaf rehydration capacity than did deciduous species, and more negative π_{tlp}, Ψ_{SC50}, and Ψ_{SC80}. These findings provide a further contributing mechanism for the dominance of evergreen leaves in areas associated with environmental stress especially given limited nutrient availability (Chabot & Hicks, 1982; Reich, Walters, & Ellsworth, 1992), which has been associated with structural and compositional differences, for example, denser cells and thicker cell walls (John et al., 2017; Villar, Robleto, De Jong, & Poorter, 2006), and higher modulus of elasticity (ε; Bartlett, Scoffoni, & Sack, 2012).

We investigated the structural and functional trait correlates of leaf rehydration capacity. Across species, the RWC and Ψ_{leaf} at turgor loss point correlated with those at $PLRC_{10}$. However, we found no relationship between indices of PLRC and π_o, thus providing no evidence that a high osmotic concentration acts to protect cytoplasm under stress. More sensitive species tended to have higher ε, which minimizes cell volume shrinkage as soil and leaf water potential fall (Bartlett, Scoffoni, & Sack, 2012), possibly reducing the need for dehydration-tolerant cells. Additionally, across species, rehydration capacity was linked with gross leaf dimensions and composition, that is, smaller leaf area and greater LT, LD, LMA, and SWC. Future work is needed to resolve how these traits might contribute causally to PLRC or are independently coselected during adaptation to drought. If these associations between PLRC and easily measured functional traits prove general, they could enable rapid estimation of leaf dehydration tolerance.

4.5 | Comparisons across ecosystems based on compiled published data

In compiled data from 17 papers for 89 species representing four ecosystems, we found ranges and means for $RWC@PLRC_{10}$, $RWC@PLRC_{25}$, and $RWC@PLRC_{50}$ comparable with those of our 18 Southern California species. Species differed considerably in their $RWC@PLRC_{10}$ across ecosystems. Species native to rainforests and Mediterranean woodlands had the most sensitive leaves, whereas Mediterranean chaparral, temperate forest, and grassland species had lower $RWC@PLRC_{10}$ on average. A likely explanation for this pattern relates to the incidence of extreme dehydration during the growing season, with species of systems facing occasional or seasonal drought during the growing season (i.e., temperate forests and grasslands) showing greater potential to recover under severe dehydration.

By contrast, Mediterranean chaparral plants have growing seasons typically in wet late spring and early summer and can be dormant during the dry summer, with closed stomata and slow rates of dehydration, or even shedding leaves (Jacobsen, Pratt, Davis, & Ewers, 2007). Finally, plants of Mediterranean woodlands typically inhabit riparian zones with year-round water supply, and similarly, tropical wet forests have typically wet soil; both would experience low selective pressure for leaf dehydration tolerance.

In the global data compilation, as in the California study species, there were no significant differences in leaf rehydration capacity between trees or shrubs, but grasses had significantly greater capacity to rehydrate than had other growth forms. Contrary to our study species, in the global data compilation, we found no significant differences in leaf dehydration tolerance by leaf habit, potentially due to the stronger functional variation within than among evergreen and deciduous leaves across diverse ecosystems and/or from discrepancies in methodology across many studies.

4.6 | Future directions in determining leaf viability

Future study is needed to identify the mechanisms for species differences in PLRC thresholds. Although classical work has shown the correspondence of PLRC to other indices of cell failure and mortality, PLRC must be related to other indices of recovery including gas exchange capacity, chlorophyll fluorescence, and hydraulic conductance. Notably, although PLRC was determined over the time course of 8 hr, and our experiments showed that for the species tested this time was sufficient to reflect what would occur with 24 hr of rehydration, it is possible that additional capacity could be recovered over longer time periods after rewatering of a plant, (Rychnovská-Soudková, 1963). Yet the loss of leaf function reflected in >10% PLRC would be expected to result in a significant reduction of photosynthetic performance and yield in at least the short term, a meaningfully negative impact, that remains to be tested. We note also that the drought hardening imposed prior to experiments in this study, to enable quantification of drought responses as experienced in the field, that is, given time to acclimate (Ruiz-Sánchez, Domingo, Torrecillas, & Pérez-Pastor, 2000; Yang, Chen, Wang, & Gong, 2015), may have led to some loss of rehydration capacity even before this was quantified or, alternatively, acclimation enabling greater rehydration capacity. Thus, studies are needed to examine if repeated leaf dehydration and rehydration would lead to accumulation of PLRC, or, alternatively, if stressed tissues increase their abilities to recover from future dehydration episodes.

The loss of rehydration capacity also has important implications for the interpretation of pressure–volume curves. Previous work has indicated that pressure–volume curve parameters may be altered due to solute redistribution during rehydration (Meinzer, Rundel, Sharifi, & Nilsen, 1986), and due to osmotic and elastic adjustments (White, Beadle, & Worledge, 1996). Our results suggest that PLRC would also influence pressure–volume curve parameters: The RWC of a leaf of a given water potential may be substantially higher if the leaf has been dehydrated and rehydrated, given decreased SWC after rehydration relative to original pre-dehydration SWC. Future work is needed to evaluate these effects for leaves operating in natural systems.

An important direction of future work is to more precisely link PLRC thresholds with the bulk water status of the cells actually experiencing the drought in the individualized tissues of the leaf. Importantly, there is a need to distinguish the water in the apoplast versus symplast. The symplastic RWC threshold for given levels of PLRC can be calculated on the basis of estimates of apoplastic fraction, though these are subject to uncertainty (Sack et al., 2017). Further, for strongly dehydrated leaves, in which embolism forms in the xylem, the apoplastic fraction will change, especially when large proportions of midrib water are lost, as this can account for up to >40% of leaf water in some species with exceptional midrib xylem volume per leaf area (unpublished data), which confers a level of uncertainty to the meaning of PLRC in highly dehydrated leaves, that is, $PLRC_{25}$ and $PLRC_{50}$.

Given that leaf water status thresholds for PLRC correlate across ecosystems with drought adaptation and correlate with other drought tolerance traits, it is likely that rehydration capacity is adaptive and important among traits determining variation in overall drought tolerance across and within species, in addition to traits that delay dehydration and/or facilitate rehydration, such as rooting depth, turgor loss point, stomatal closure, resistance to xylem embolism, and capacity for foliar water uptake.

Finally, we suggest that quantifying rehydration capacity would have applications toward achieving greater precision in estimating leaf water content or water potential from LT in crop and forest monitoring and irrigation design (Afzal, Duiker, & Watson, 2017). Previous work has found hysteresis in experiments using LT as an index of water content (Buquez, 1987), consistent with our finding that tissues do not fully recover in water content (and thus cell volumes and LT) after rehydration. The ability to account for losses of rehydration capacity as a function of dehydration would thus improve the ability to estimate RWC from LT as it varies over the courses of minutes and hours and during seasonal cycles of dehydration and rehydration. Notably, further studies are equally needed of long-scale responses of leaves on droughted plants, weeks or months after recovery from water deficits (Anderegg et al., 2013; Brodribb et al., 2010), to determine whether leaves not shed can recover further rehydration capacity and function, or whether they remain only partially intact but dysfunctional, for species of a range of leaf habits that vary in other parameters (e.g., Skelton, Brodribb, McAdam, & Mitchell, 2017).

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AUTHOR CONTRIBUTION

G.J. and L.S. designed the study, C.H. provided stomatal closure data, G.J. collected all other data, and G.J. and L.S. wrote the paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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